Protein refolding from pellet samples

Introduction

After cell lysis and centrifugation the possibly misfolded proteins can be refolded with this protocol

Materials

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Procedure

Protein refolding

1. Resuspend the samples in 50 mM Tris-HCl at pH 7.4, 4 M urea
2. Sonicate samples for four minutes, 5 sec on, 5 sec off, at 30 % Amp to wash
3. Centrifuge 12 000 g for 10 min, RT
4. Collect supernatant
5. Repeat the washing three times, collect supernatant
6. Incubate the supernatant at 37 °C for minimum 1 hour
7. If your sample is cloudy after this add 8 M urea until it becomes clear, incubate for 1 h at 37 °C
8. Dialyze your denaturated protein samples first at a 1:10 ratio against buffer containing 10 % v/v glycerol and 0.1 mM EDTA (refolding buffer), 20 mM HEPES pH 7.4 at 4 °C for 4 h
9. Optional: Concentrate your sample
10. Second dialysis with a 1:100 ratio against the same buffer at 4 °C for 16 h or O/N
11. Concentrate samples for further analysis